Cell Surface Engineering for the Biology / Materials Interface

Second, Independent, Specific Linkage Developed

A major impediment to the development of hybrid living / non-living materials and systems is the design of methods to maintain the stability of the interface between the components. Mechanisms must be found to allow living cells to be attached to inorganic materials without compromising either the unique life functions of the cells or the structural and functional integrity of the materials.

As reported in a recent issue of *Science*, Carolyn Bertozzi and her colleagues, as part of their CAM Biomolecular Materials program studying the "materials-biology interface," have developed their second technique to engineer the surfaces of human cells so that they can be attached to a variety of materials surfaces without loss of function (see MSD Highlight 99-10). As in the first case, the general principle involves providing the cells with designed chemical variants of their natural nutrients and allowing them to process those "pseudo nutrients" and attach them to the ends of the complex carbohydrates that cover their surfaces. The method depends on the ability to design "pseudo nutrients" 1) that are not toxic to the cell, 2) that the cell can incorporate, transform, and place on their surfaces, and 3) that can react easily with the desired materials surfaces without damaging the materials or the cells. Further, these molecules must be different from the molecules that are naturally found on the cell surface, so attachment can be specific and controllable.

The new system is based on the "Staudinger" reaction that occurs between an azide, which is to be placed on the cell surface, and a phosphine, which is to be placed on the materials surface. The reaction links the two in a compound called an aza-ylide (see figure). As required for the goals of this project, neither phosphines nor azides are found on the surface of living cells and they do not react with biological molecules. They do, however, react rapidly with each other, forming a linkage with high efficiency in water and at room temperature. Unfortunately, however, the resulting aza-ylide is unstable in water and quickly hydrolyzes, breaking the cell-material bond. To solve this problem the group developed new chemistry modifying the phosphine with the addition of a "methyl ester" group which, rather than allowing disruptive hydrolysis of the linker molecule, causes a rearrangement of the aza-ylide to a stable "amide" bond.

To place the azides onto the cell surface, the group fed growing cells a normal nutrient, N–acylmannosamine, to which an azide group had been attached (see figure). The cells ignored the azide group and treated the variant as they do unmodified N-acetylmannosamine, processing it to sialic acid and then incorporating that sialic acid into the carbohydrate on the cell surface. To demonstrate that the azide-laden sialic acid was in fact transported to the surface, the cells were exposed to fluorescently labeled phosphines. As predicted, the cells soon became brightly fluorescent, as the labeled phosphine bound the azide. Cells treated with unmodified sialic acid without the azide did not fluoresce. Modified cells cultured for several days showed no change in growth rate, indicating that neither the artificial sugars nor the attachment of the fluorescent probes affected their viability. The azide-labeled cells are thus primed to attach to other materials and components of composite devices to which phosphines have been attached.

This new method extends the earlier reported work in which ketone markers were placed on the cell's surface. These react with hydrazides placed on the surface of materials to link the cells to those materials. With two such independent and specific linkages, multiple specific attachments to different materials can be achieved. As a "spin-off" of this work, the azide labeling of cancer cells can be used to target phosphine labeled anticancer agents directly and specifically to their targets, sparing normal cells.

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Eliana Saxon and C.R. Bertozzi, "Cell Surface Engineering by a Modified Staudinger Reaction," *Science*, 2000 March 17; 287: 2007-1010.